

AMENDMENTS TO THE CLAIMS

The following is a listing of claims that replaces all prior versions, and listings, of claims in the application. Underlining denotes added text, and strikethrough denotes cancelled text.

1. (Previously Amended) A method of identifying a test agent that modulates at least one activity selected from the group consisting of microtubule depolymerization, microtubule polymerization and microtubule severing, said method comprising the steps of:
 - (i) contacting a polymerized microtubule with at least one protein selected from the group consisting of a microtubule severing protein and a microtubule depolymerizing protein, in the presence of ATP or GTP, and said test agent; and
 - (ii) detecting the formation of at least one product selected from the group consisting of tubulin monomers, dimers and oligomers, wherein the formation of said tubulin monomers, dimers, or oligomers indicates that said test agent modulates microtubule depolymerization.
2. (Previously Amended) The method of claim 1, wherein said polymerized microtubule is labeled with 4'-6-diamidino-2-phenylindole (DAPI).
3. (Original) The method of claim 1, wherein said detecting is by fluorescent resonance energy transfer (FRET).
4. (Original) The method of claim 2, wherein said detecting, comprising detecting a change in fluorescence of said labeled microtubule.
5. (Original) The method of claim 1, wherein said detecting comprises centrifuging said tubulin monomers if present.

6. (Original) The method of claim 1, wherein said microtubules are stabilized by contact with an agent selected from the group consisting of paclitaxel, a paclitaxel analogue, and a non-hydrolyzable nucleotide GTP analogue.

7. (Original) The method of claim 1, wherein said microtubule is attached to a solid surface.

8. (Original) The method of claim 7, wherein said microtubule is attached to said surface by binding with an agent selected from the group consisting of an inactivated microtubule motor protein, an avidin-biotin linkage, an anti-tubulin antibody, a microtubule binding protein (MAP), and a polylysine.

9. (Previously Amended) The method of claim 1, wherein said microtubule severing protein or microtubule depolymerizing protein is selected from the group consisting of katanin polypeptide, p60 subunit of katanin polypeptide, *Xenopus* kinesin central motor 1 (XKCM1) polypeptide, and stathmin (OP18) polypeptide.

10. (Previously Amended) The method of claim 9, wherein said microtubule severing protein is katanin polypeptide or p60 subunit of katanin polypeptide.

11. (Previously Amended) The method of claim 10, wherein said p60 subunit of a katanin is a polypeptide having microtubule severing activity, wherein said polypeptide comprises an isolated p60 subunit of katanin, and wherein said p60 subunit is encoded by a nucleic acid that hybridizes with a nucleic acid encoding the amino acid of SEQ ID NO:1, when incubated at 42°C overnight in 50% formamide.

12. (Original) The method of claim 10, wherein said p60 subunit is a polypeptide having the amino acid sequence of SEQ ID NO:1.

13. (Original) The method of claim 1, wherein said method is performed in an array where said array comprises a multiplicity of reaction mixtures, each reaction mixture

comprising a distinct and distinguishable domain of said array, and wherein said steps are performed in each reaction mixture.

14. (Original) The method of claim 13, wherein said array comprises a microtitre plate.

15. (Original) The method of claim 13, wherein said array comprises at least 48 of said reaction mixtures.

16. (Previously Amended) The method of claim 13, wherein said test agent is one of a plurality of test agents and wherein each reaction mixture comprises one test agent of said plurality of test agents.

17. (Previously Amended) A method of identifying a therapeutic lead compound that modulates at least one activity selected from the group consisting of depolymerization, polymerization, and severing of a microtubule system, said method comprising the steps of:

- i) providing an assay mixture comprising a katanin p60 subunit and a microtubule;
- ii) contacting said assay mixture with a test agent to be screened for the ability to inhibit or enhance the microtubule severing or ATPase activity of said p60 subunit; and
- iii) detecting at least one of specific binding of said test compound to said p60 subunit and a change in the ATPase activity of said p60 subunit.

18. (Original) The method of claim 17, wherein said detecting comprises detecting ATPase activity utilizing malachite green as a detection reagent.

19. (Original) The method of claim 17, wherein said p60 subunit is labeled and said test agent is attached to a solid support.

20. (Original) The method of claim 17, wherein said test agent is labeled and said p60 subunit is attached to a solid support.

21. (Previously Amended) The method of claim 17, wherein said microtubules are stabilized by contact with an agent selected from the group consisting of paclitaxel, a paclitaxel analogue, and a non-hydrolyzable nucleotide GTP analogue.

22. (Original) The method of claim 17, wherein said method is performed in an array where said array comprises a multiplicity of reaction mixtures, each reaction mixture comprising a distinct and distinguishable domain of said array, and wherein said steps are performed in each reaction mixture.

23. (Original) The method of claim 22, wherein said array comprises a microtitre plate.

24. (Original) The method of claim 22, wherein said array comprises at least 48 of said reaction mixtures.

25. (Previously Amended) The method of claim 22, wherein said test agent comprises one of a plurality of test agents and wherein each reaction mixture comprises one test agent of said plurality of test agents.

Claims 26-42 (Previously Canceled)

Claims 43-46 (Currently Cancelled).

Claim 47 (Previously Cancelled).

Claims 48-51 (Currently Cancelled).

52. (Previously Amended) The method of claim 51, wherein said microtubule severing protein is a p60 subunit of a katanin.

53. (Previously Amended) The method of claim 52, wherein said p60 subunit of a katanin is a polypeptide having microtubule severing activity, wherein said polypeptide comprises an isolated p60 subunit of katanin, and wherein said p60 subunit is encoded by a nucleic acid that hybridizes with a nucleic acid encoding the amino acid of SEQ ID NO:1, when incubated at 42°C overnight in 50% formamide.

Claims 54-59 (Currently Cancelled).

60. (Previously Added) A method of screening for a test agent that alters at least one activity selected from the group consisting of microtubule polymerization, microtubule depolymerization, and microtubule severing, said method comprising:

- a) providing:
 - i) labeled tubulin,
 - ii) an isolated polypeptide having at least one activity selected from the group consisting of microtubule polymerization activity, microtubule depolymerization activity, and microtubule severing activity, said polypeptide comprising a katanin p60 subunit, and
 - iii) a test agent;
- b) contacting said labeled tubulin with said isolated polypeptide and with said test agent to produce contacted tubulin; and
- c) comparing the fluorescence intensity or pattern of said contacted tubulin with the fluorescence intensity or pattern of labeled tubulin that is not contacted with said polypeptide and said test agent, wherein a difference in fluorescence pattern or intensity between the contacted and the not contacted tubulin indicates that said test agent alters at least one activity selected from the group consisting of microtubule polymerization, microtubule depolymerization, and microtubule severing.

61. (Previously Added) The method of Claim 60, wherein said labeled tubulin is in at least one form selected from the group consisting of tubulin monomers, tubulin dimers, and tubulin oligomers.

62. (Previously Added) The method of Claim 60, wherein said labeled tubulin is in the form of a microtubule.

63. (Previously Added) The method of Claim 62, wherein said microtubule is attached to a solid surface.

64. (Previously Added) The method of Claim 63, wherein said microtubule is attached to said surface by binding with a molecule selected from the group consisting of an inactivated microtubule motor protein, an avidin-biotin linkage, an anti-tubulin antibody, a microtubule binding protein (MAP), a polyarginine, a polyhistidine, and a polylysine.

65. (Previously Added) The method of Claim 62, wherein the label of said labeled tubulin is selected from the group consisting of 4'-6-diamidino-2-phenylindole (DAPI), anilinonapthalene sulfonate (ANS), bis-ANS (Bis-anilinonapthalene sulfonate), N-phenyl-1-naphthylene (NPN), ruthenium red, cresol violet, and 4-(dicyanovinyl)julolidine (DCVJ).

66. (Previously Added) The method of Claim 62, wherein the label of said labeled tubulin is 4'-6-diamidino-2-phenylindole (DAPI).

67. (Previously Added) The method of Claim 60, wherein said katanin p60 subunit is recombinant.

68. (Previously Added) The method of Claim 60, wherein said katanin p60 subunit has the amino acid sequence of SEQ ID NO:1.

69. (Previously Added) The method of Claim 60, wherein said method is performed in an array, wherein said array comprises a multiplicity of reaction mixtures.

70. (Previously Added) The method of Claim 69, wherein said array comprises a microtitre plate.

71. (Previously Added) The method of Claim 69, wherein said array comprises at least 48 of said reaction mixtures.

72. (Previously Added) The method of Claim 60, further comprising step d) listing the test agents that alter at least one of microtubule polymerization, microtubule depolymerization, and microtubule severing into a database.

73. (Previously Added) A method of screening for a test agent that alters at least one activity selected from the group consisting of microtubule polymerization, microtubule depolymerization, and microtubule severing, said method comprising:

- a) providing:
 - i) labeled tubulin,
 - ii) an isolated katanin p60 subunit, and
 - iii) a test agent;
- b) contacting said labeled tubulin with said isolated katanin p60 subunit and with said test agent to produce contacted tubulin; and
- c) comparing the fluorescence intensity or pattern of said contacted tubulin with the fluorescence intensity or pattern of labeled tubulin that is not contacted with said polypeptide and said test agent, wherein a difference in fluorescence pattern or intensity between the contacted and the not contacted tubulin indicates that said test agent alters at least one activity selected from the group consisting of microtubule polymerization, microtubule depolymerization, and microtubule severing.

74. (Previously Added) The method of Claim 73, wherein said labeled tubulin is in at least one form selected from the group consisting of tubulin monomers, tubulin dimers, and tubulin oligomers.

75. (Previously Added) The method of Claim 73, wherein said labeled tubulin is in the form of a microtubule.

76. (Previously Added) The method of Claim 75, wherein said microtubule is attached to a solid surface.

77. (Previously Added) The method of Claim 76, wherein said microtubule is attached to said surface by binding with a molecule selected from the group consisting of an inactivated microtubule motor protein, an avidin-biotin linkage, an anti-tubulin antibody, a microtubule binding protein (MAP), a polyarginine, a polyhistidine, and a polylysine.

78. (Previously Added) The method of Claim 75, wherein the label of said labeled tubulin is selected from the group consisting of 4'-6-diamidino-2-phenylindole (DAPI), anilinonapthalene sulfonate (ANS), bis-ANS (Bis-anilinonapthalene sulfonate), N-phenyl-1-naphthylene (NPN), ruthenium red, cresol violet, and 4-(dicyanovinyl)julolidine (DCVJ).

79. (Previously Added) The method of Claim 75, wherein the label of said labeled tubulin is 4'-6-diamidino-2-phenylindole (DAPI).

80. (Previously Added) The method of Claim 73, wherein said katanin p60 subunit is recombinant.

81. (Previously Added) The method of Claim 73, wherein said katanin p60 subunit has the amino acid sequence of SEQ ID NO:1.

82. (Previously Added) The method of Claim 73, wherein said method is performed in an array, wherein said array comprises a multiplicity of reaction mixtures.

83. (Previously Added) The method of Claim 82, wherein said array comprises a microtitre plate.

84. (Previously Added) The method of Claim 82, wherein said array comprises at least 48 of said reaction mixtures.

85. (Previously Added) The method of Claim 73, further comprising step d) listing the test agents that alter at least one of microtubule polymerization, microtubule depolymerization, and microtubule severing into a database.

86. (Previously Added) A method of screening for a test agent that alters at least one activity selected from the group consisting of microtubule polymerization and depolymerization, said method comprising:

- a) providing labeled tubulin;
- b) contacting said labeled tubulin with said test agent to produce contacted tubulin; and
- c) comparing the fluorescence intensity or pattern of said contacted tubulin with the fluorescence intensity or pattern of labeled tubulin that is not contacted with said test agent wherein a difference in fluorescence pattern or intensity between the contacted and the not contacted tubulin indicates that said test agent alters at least one activity selected from the group consisting of microtubule polymerization and depolymerization.

87. (Previously Added) The method of claim 86, wherein said labeled tubulin is in at least one form selected from the group consisting of tubulin monomers, tubulin dimers, and tubulin oligomers.

88. (Previously Added) The method of claim 86, wherein said labeled tubulin is in the form of a microtubule.

89. (Previously Added) The method of claim 88, wherein said microtubule is attached to a solid surface.

90. (Previously Added) The method of claim 88, wherein the label of said labeled tubulin is selected from the group consisting of 4'-6-diamidino-2-phenylindole (DAPI), anilinonapthalene sulfonate (ANS), bis-ANS (Bis-anilinonapthalene sulfonate), N-phenyl-1-naphthylene (NPN), ruthenium red, cresol violet, and 4-(dicyanovinyl)julolidine (DCVJ).

91. (Previously Added) The method of claim 90, wherein said label is 4'-6-diamidino-2-phenylindole (DAPI).

92. (Previously Added) The method of claim 89, wherein said microtubule is attached to said surface by binding with an agent selected from the group consisting of an inactivated microtubule motor protein, an avidin-biotin linkage, an anti-tubulin antibody, a microtubule binding protein (MAP), a polyarginine, a polyhistidine, and a polylysine.

93. (Previously Added) A method of screening for a test agent that alters at least one activity selected from the group consisting of microtubule polymerization and depolymerization, said method comprising:

- a) providing:
 - i) labeled tubulin,
 - ii) a microtubule depolymerizing protein, and
 - iii) a test agent;
- b) contacting said tubulin with said microtubule depolymerizing protein and with said test agent to produce contacted tubulin; and
- c) comparing the fluorescence intensity or pattern of said contacted tubulin with the fluorescence intensity or pattern of labeled tubulin that is not

contacted with said test agent, wherein a difference in fluorescence pattern or intensity between the contacted and the not contacted tubulin indicates that said test agent alters at least one activity selected from the group consisting of microtubule polymerization and depolymerization.

94. (Previously Added) The method of claim 93, wherein said microtubule depolymerizing protein comprises a *Xenopus* kinesin central motor 1 (XKCM1) polypeptide.

95. (Previously Added) The method of claim 86, wherein said method is performed in an array where said array comprises a multiplicity of reaction mixtures, and wherein said steps are performed in each reaction mixture.

96. (Previously Added) The method of claim 95, wherein said array comprises a microtitre plate.

97. (Previously Added) The method of claim 95, wherein said array comprises at least 48 of said reaction mixtures.

98. (Previously Added) The method of claim 95, wherein said test agent comprises a plurality of test agents and wherein each reaction mixture comprises one test agent of said plurality of test agents.

99. (Previously Added) The method of claim 86, further comprising listing the test agents that alter at least one of microtubule polymerization and depolymerization into a database of therapeutic lead compounds that act on the cytoskeletal system.

100. (Previously Added) The method of claim 93, wherein said labeled tubulin is in at least one form selected from the group consisting of tubulin monomers, tubulin dimers, and tubulin oligomers.

101. (Previously Added) The method of claim 93, wherein said labeled tubulin is in the form of a microtubule.

102. (Previously Added) The method of claim 101, wherein said microtubule is attached to a solid surface.

103. (Previously Added) The method of claim 101, wherein the label of said labeled tubulin is selected from the group consisting of 4'-6-diamidino-2-phenylindole (DAPI), anilinonaphthalene sulfonate (ANS), bis-ANS (Bis-anilinonaphthalene sulfonate), N-phenyl-1-naphthylene (NPN), ruthenium red, cresol violet, and 4-(dicyanovinyl)julolidine (DCVJ).

104. (Previously Added) The method of claim 103, wherein said label is 4'-6-diamidino-2-phenylindole (DAPI).

105. (Previously Added) The method of claim 102, wherein said microtubule is attached to said surface by binding with an agent selected from the group consisting of an inactivated microtubule motor protein, an avidin-biotin linkage, an anti-tubulin antibody, a microtubule binding protein (MAP), a polyarginine, a polyhistidine, and a polylysine.

106. (Previously Added) The method of claim 93, wherein said method is performed in an array, wherein said array comprises a multiplicity of reaction mixtures, and wherein said steps are performed in each reaction mixture.

107. (Previously Added) The method of claim 106, wherein said array comprises a microtitre plate.

108. (Previously Added) The method of claim 106, wherein said array comprises at least 48 of said reaction mixtures.

109. (Previously Added) The method of claim 106, wherein said test agent comprises a plurality of test agents, and wherein each reaction mixture comprises one test agent of said plurality of test agents.

110. (Previously Added) The method of claim 93, further comprising listing the test agents that alter at least one of microtubule polymerization and depolymerization into a database of therapeutic lead compounds that act on the cytoskeletal system.

111. (Previously Added) The method of claim 93, wherein said microtubule depolymerizing protein is a *Xenopus* kinesin central motor 1 (XKCM1) polypeptide.

112. (Previously Added) A method of screening for a test agent that alters at least one activity selected from the group consisting of microtubule polymerization and depolymerization, said method comprising:

- a) providing:
 - i) labeled tubulin,
 - ii) a microtubule depolymerizing protein comprising a stathmin polypeptide, and
 - iii) a test agent;
- b) contacting said labeled tubulin with said microtubule depolymerizing protein and with said test agent to produce contacted tubulin; and
- c) comparing the fluorescence intensity or pattern of said contacted tubulin with the fluorescence intensity or pattern of labeled tubulin that is not contacted with said test agent, wherein a difference in fluorescence pattern or intensity between the contacted and the not contacted tubulin indicates that said test agent alters at least one activity selected from the group consisting of microtubule polymerization and depolymerization.

113. (Previously Added) The method of claim 112, wherein said method is performed in an array where said array comprises a multiplicity of reaction mixtures, and wherein said steps are performed in each reaction mixture.

114. (Previously Added) The method of claim 113, wherein said array comprises a microtitre plate.

115. (Previously Added) The method of claim 113, wherein said array comprises at least 48 of said reaction mixtures.

116. (Previously Added) The method of claim 113, wherein said test agent comprises a plurality of test agents and wherein each reaction mixture comprises one test agent of said plurality of test agents.

117. (Previously Added) The method of claim 112, further comprising listing the test agents that alter at least one of microtubule polymerization and depolymerization into a database of therapeutic lead compounds that act on the cytoskeletal system.

118. (Previously Added) The method of claim 112, wherein said labeled tubulin is in at least one form selected from the group consisting of tubulin monomers, tubulin dimers, and tubulin oligomers.

119. (Previously Added) The method of claim 112, wherein said labeled tubulin is in the form of a microtubule.

120. (Previously Added) The method of claim 119, wherein said microtubule is attached to a solid surface.

121. (Previously Added) The method of claim 119, wherein the label of said labeled tubulin is selected from the group consisting of 4'-6-diamidino-2-phenylindole (DAPI), anilinonapthalene sulfonate (ANS), bis-ANS (Bis-anilinonapthalene sulfonate), N-phenyl-1-naphthylene (NPN), ruthernium red, cresol violet, and 4-(dicyanovinyl)julolidine (DCVJ).

122. (Previously Added) The method of claim 121, wherein said label is 4'-6-diamidino-2-phenylindole (DAPI).

123. (Previously Added) The method of claim 120, wherein said microtubule is attached to said surface by binding with an agent selected from the group consisting of an inactivated microtubule motor protein, an avidin-biotin linkage, an anti-tubulin antibody, a microtubule binding protein (MAP), a polyarginine, a polyhistidine, and a polylysine.

124. (Previously Added) The method of claim 112, wherein said microtubule depolymerizing protein is a stathmin polypeptide.